

Molecularly Imprinted Stationary Phases for Microfluidic Chemical Separations

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The overall purpose of the project is to synthesize molecular imprint polymer (MIP) stationary phases that are specific for one molecule. We have focused our work on the creation of open tubular-type (as opposed to packed column) stationary phases that are covalently tethered to the *plastic* microfluidic channel. The synthesis of the MIP films incorporate surface-bound alkene groups into the polymerization of methacrylic acid (MAA) in the presence of ethylene glycol dimethacrylate (EGDMA). Prior to the polymerization, a template molecule is added to the mixture. The organic functional groups on the template molecule can interact, by hydrogen bonding, with the functional groups on the MAA. After polymerization, the template molecule is extracted, leaving behind “pockets” that are specific in size and shape to the template molecule. To date, we have shown that the MIP thin films synthesized on plastic surfaces are selective for molecules similar to the template molecule. The ultimate goal of this project is to synthesize MIP stationary phases tethered to the plastic microchannel that will allow for the separation of proteins on the basis of their quaternary and tertiary structures. In addition, we would like to develop a benchtop separation system that will discriminate between racemates such as those regularly synthesized in the pharmaceutical industry.

The synthesis of the covalently-tethered MIP thin films was the first major step in this project. The synthesis involved first chemically modifying PMMA surfaces such that a

reactive functional group, primary amines, populated the surface. The primary amines were then coupled to MAA, terminating the surface in alkene functionalities. The alkenes were used in the polymerization of MAA in the presence of EGDMA to form a ~5- μm -thick, robust poly(methacrylic acid) (PMAA) surface. Analytical characterization of these thin films included such techniques as reflection-absorption infrared spectroscopy (RAIRS), water contact angle studies, and scanning electron microscopy (SEM).

Displayed in Figure 1 are infrared spectra of amine-terminated PMMA, alkene-terminated PMMA, and PMAA-

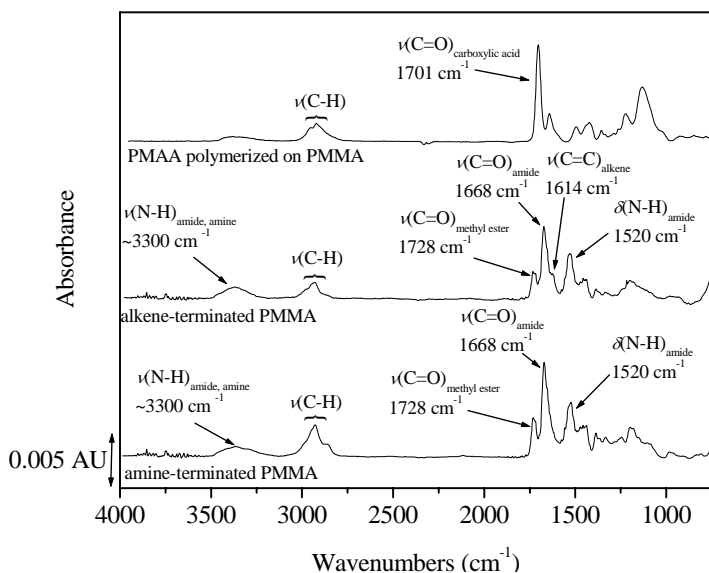


Figure 1. Reflection-absorption infrared spectra of amine-, alkene-, and PMAA-terminated PMMA thin films spin-coated on Au.

terminated PMMA. The RAIR spectrum matching the amine-terminated PMMA contains bands corresponding to an amide linkage to a primary amine on the surface of the PMMA. In the case of alkene-terminated PMMA, many of the same bands that are found on the amine-terminated PMMA RAIR spectrum are noted; in addition, a band at 1614 cm^{-1} , corresponding to $\nu(\text{C}=\text{C})_{\text{alkene}}$, is noted. This band suggests the presence of a terminal alkene on the surface of the PMMA. The band is no longer evident on the RAIR spectrum corresponding to the PMAA polymerized on PMMA, suggesting that the terminal alkene was involved in the polymerization. A band at 1701 cm^{-1} , corresponding to $\nu(\text{C}=\text{O})_{\text{carboxylic acid}}$, is apparent.

Two fluorophores with similar structures were used as template molecules for the fabrication of MIP thin films. Three films were synthesized: (1) an MIP film that employed carboxyfluorescein mixed isomers as the template molecule, (2) an MIP film that employed 2', 7' difluorofluorescein (Oregon Green) as the template molecule, and (3) a film that contained no template molecules. The template molecules were extracted into pH 9 carbonate buffer and the three films were exposed to 1 nmol/L Oregon Green in pH 7 phosphate buffer for a fixed amount of time and rinsed. The films were then

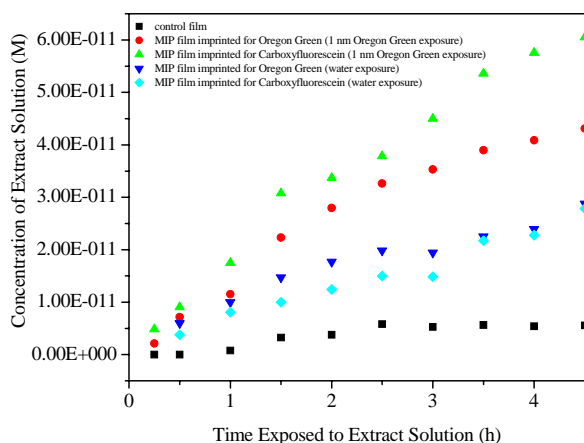


Figure 2: Extraction of Oregon Green from MIP matrix after exposure to 1 nM Oregon Green in pH 7 phosphate buffer.

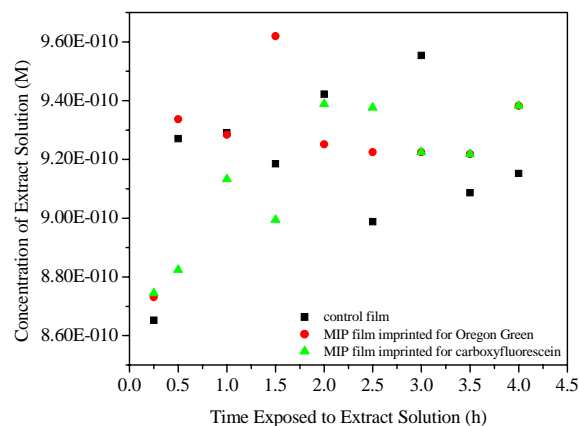


Figure 3: Extraction of Rhodamine B from MIP matrix after exposure to 1 nM Rhodamine B in pH 7 phosphate buffer.

extracted over time in pH 9 carbonate buffer and analyzed by fluorescence spectroscopy, Figure 2. As is readily noted, the carboxyfluorescein-imprinted thin film seems slightly more specific for the Oregon Green molecule. This may be attributed to the structure similarities of the two molecules. When the films were exposed to Rhodamine B and analyzed, Figure 3, no trend in concentration was evident, implying that all surface interactions were nonspecific.

Future work will involve improving the selectivity of the MIP thin films as well as using the thin films in a separation of chiral compounds. Proteins and other biologically important molecules will also be incorporated as template molecules.